DASHEEN MOSAIC VIRUS OF CULTIVATED AROIDS AND ITS CONTROL BY SEED PROPAGATION AND CULTURE OF SHOOT TIPS

By

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Veil your face, O foolish experimenter, overconfident in your mischievous straw! You thought that you had created a new type of instrumentalist; and you have obtained nothing at all. The Cricket has thwarted your schemes: he is scraping with his right fiddlestick and always will. Your sorry science tried to make a left-handed player of him. He laughs at your devices and settles down to be right-handed for the rest of his life.

-- J. Henri Fabre

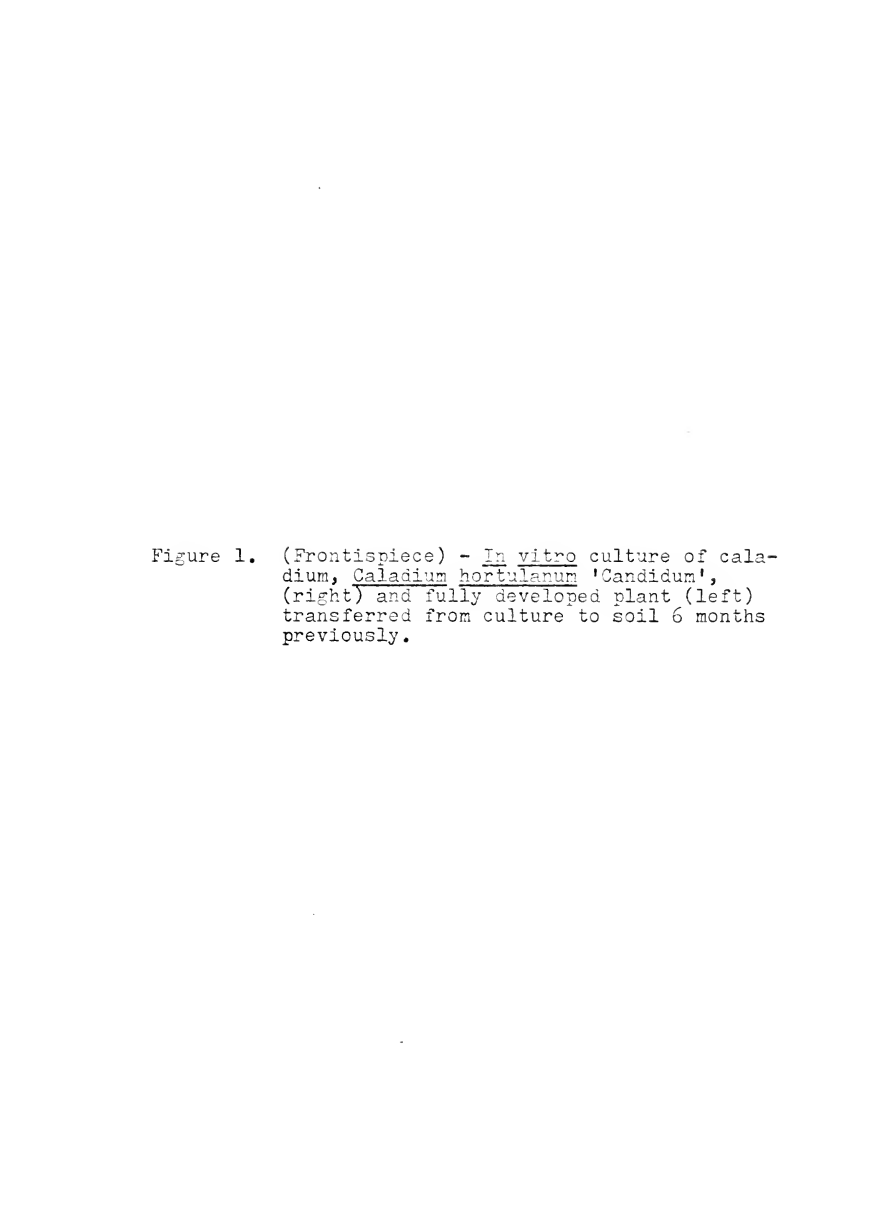

Figure 1. (Frontispiece) - *In vitro* culture of caladium, *Caladium hortulanum* 'Candidum', (right) and fully developed plant (left) transferred from culture to soil 6 months previously.

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DASHEEN MOSAIC VIRUS OF CULTIVATED AROIDS AND ITS CONTROL BY SEED PROPAGATION AND CULTURE OF SHOOT TIPS

By

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Chairman: Dr. F. W. Zettler
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Dasheen mosaic virus (DMV) was recovered from species representing 13 genera in the Araceae, but did not infect seedlings of 45 species in 17 other families. The following results of this investigation support earlier reports that DMV is a member of the "potato virus Y" group of plant viruses: 1) a mean particle length of 782 nm was recorded, 2) characteristic cylindrical inclusions were observed and 3) the virus proved serologically related to potato Y and tobacco etch viruses. Surveys indicate that DMV is prevalent in commercial plantings of aroids and induces serious losses. Infected plants were encountered in all Caladium hortulanum (caladium), Colocasia esculenta (taro) and Xanthosoma sagittifolium (cocoyam) fields surveyed in Florida and in all stock beds of Dieffenbachia picta (dieffenbachia) in ornamental foliage plant nurseries visited. Based on these surveys, it appears that DMV has become uniformly established in certain cultivars of caladium and dieffenbachia.

The deleterious effects of DMV on yields of caladium, dieffenbachia, Philodendron selloum and Zantedeschia elliottiana were assessed in controlled experiments. Weight losses of 40, 63, 30 and 59% and reductions in leaf area of 53, 66, 30 and 51% were recorded, respectively. Virus-free plants of caladium, dieffenbachia, and Zantedeschia spp. were obtained through seed propagations, although caladium and dieffenbachia progeny differed from parental plants. Excised shoot-tips of caladium, cocoyam and taro were successfully cultured aseptically and free of DMV on the revised medium of Murashige and Skoog. Callus of all three species proliferated into numerous plantlets on this medium and plantlets survived transplanting from culture to soil. Caladium plantlets assumed characteristic foliar variegation within three months after transplanting and attained maturity within 6-7 months.

INTRODUCTION

The family Araceae includes about 105 genera and 1400-1500 species of plants which are primarily of tropical or subtropical distribution, although several species occur in temperate climates. Species in eight genera are indigenous to the United States. The typical aroid inflorescence is a spadix subtended by a large, often showy spathe. These plants are mostly monoecious and dichogamous.

Many aroids are economically important. In Florida, species of Aglaonema, Dieffenbachia, Philodendron, Scindapsus and Syngonium account for over half of a $25,700,000/year foliage industry (16). Approximately 96% of the world's commercially produced caladiums are also grown in Florida (39). Cryptocoryne spp. are of considerable significance to the aquarium plant industry of Florida, and another aquatic aroid, Pistia stratiotes, ranks as one of the world's most noxious waterweeds.

On an international basis, perhaps the most significant aroid genera are those containing edible species. The cocoyams (Xanthosoma spp.) and taros (Colocasia spp.) are carbohydrate staples in many tropical and subtropical areas throughout the world. In Florida approximately 3000 acres of the former are produced for the Cuban populace (49,54). Of lesser

importance as food crops are species of Alocasia, Amorphophallus, and Cyrtosperma. These are grown principally in Japan, parts of Southeast Asia, and in the South Pacific.

Most horticulturally important aroids are propagated exclusively by vegetative means. Many aroid species are sterile (51) and Hill described taro as having "been cultivated for so long that it never flowers" (38). A few aroids, however, are readily seed propagated including some species of Aglaonema, Anthurium and Philodendron. For many aroids, special techniques must be employed to achieve fertilization (46). Since, however, most ornamental aroids are heterozygous cultivars, progeny do not necessarily reflect parental types (46,71).

Continued vegetative propagation has enabled certain phytopathogens to become prevalent in stock plantings of many aroids. Viruses can become especially serious under these circumstances and indeed infections of tomato spotted wilt virus in Zantedeschia spp. and cucumber mosaic virus in Arum italicum are well documented (22,45,66,67). Virus-like diseases have also been reported in species of Amorphophallus (10,12), Anthurium (34,35,70), Philodendron (65) and P. stratiotes (53). In addition, a virus causing the "chirke disease" of large cardamon was found to be sap-transmissible to the aroid Acorus calamus (56).

In 1970, Zettler et al. (74,75) reported a filamentous virus infecting Colocasia esculenta in Florida which proved mechanically transmissible to seedlings of Philodendron

selloum and Zantedeschia elliottiana. Unlike the filamentous anthurium virus described by Herold, it failed to infect tobacco. This virus was designated as dasheen mosaic virus (DMV) and was characterized as belonging to the Potato Y virus group of Brandes (7) and Brandes and Bercks (8) in: (i) being aphid transmitted in a stylet-borne manner by aphids, (ii) having a mean particle length of 730 nm, and (iii) inducing characteristic cylindrical inclusions. An isolate of DMV was later characterized by Alconero (1) as having a (i) dilution end point between 1/100 and 1/1000, (ii) thermal inactivation point in 10 minutes between 60 and 65 C and (iii) longivity in vitro of 75 hours at 26 C.

Since its discovery in 1970, DMV has been reported from Zantedeschia spp. in the Netherlands (69) and from taro and/or cocoyam in the British Solomon Islands (23,40,41,42), Trinidad (42), Venezuela (17), Puerto Rico (2), Hawaii (2), the Fiji Islands (32) and Japan (3). In addition, a mosaic disease of Philodendron selloum in California (65) is assumed to be induced by DMV (H. R. Hill, personal communication).

Two bacilliform viruses of taro have been described in the British Solomon Islands (23,40,42). These viruses in combination with DMV reportedly kill infected plants causing a condition referred to locally as "alomae". Unlike DMV, however, neither bacilliform virus has been detected outside the island of Malaita. Alomae-infected plants were not detected elsewhere in the Protectorate, nor were they observed in material from Trinidad or Ghana.

The purpose of this study was to further characterize DMV, assess its distribution and importance among aroids in Florida and to investigate practical control measures for this virus. Attempts were also made to identify other viruses of aroids in Florida. Portions of this dissertation have been published elsewhere (26,27,28,29,30,31).

MATERIALS AND METHODS

Characterization of Dasheen Mosaic Virus

Host-range Determinations

All host range determinations were based upon manual transmission trials using inoculum expressed from symptomatic leaves and diluted with deionized water. Six hundred mesh carborundum was used as an abrasive. Unless otherwise noted, all test plants were grown from seed. Two or more leaves were inoculated per test plant. At least 4 P. selloum seedlings were inoculated after all host range trials to insure that the inoculum used was infectious, except in one instance when two caladium seedlings were used subsequent to inoculation of Rhoeo discolor. Test plants were maintained for at least 14 days following inoculations. Regardless of symptom expression, attempts were made to recover virus from test plants. In recovery trials, at least 3 P. selloum seedlings were rubbed with extracts from inoculated leaves and with extracts from leaves which developed subsequent to inoculation.

Whereas aroids were tested at various times throughout the year, all non-aroids were inoculated August-October when greenhouse temperatures were 20-30 C. At least 5 individuals of each plant species were inoculated except that only 4, 4

and 2 plants of Nicotiana sylvestrus, N. glutinosa and N. clevelandii were used, respectively.

Transmission Electron Microscopy

All plant material was examined using a Philips Model 200 electron microscope operating with an accelerating voltage of 60 KV. Size determinations were made by comparing projected electron micrographs with projected micrographs of a 54,864 line/inch diffraction grating.

Leaf extracts

All material used for examining virus particle morphology was obtained from negatively stained leaf extract preparations. Mounts were prepared by dicing a small piece of symptomatic tissue (ca. $3mm^2$) in several drops of 1-2% phosphotungstic acid (PTA), pH 6.8, contained on a glass slide. The diced tissue was mixed briefly with the PTA and a droplet of the mixture was placed on a specimen grid coated with Formvar and strengthened with arc-vaporized carbon. After one minute, excess fluid was absorbed with filter paper. Specimens were then examined with the electron microscope.

A similar procedure was used for preparing virus-induced cytoplasmic inclusions for examination except that leaf extracts were stained in 1% ammonium molybdate, pH 6.5, rather than PTA (55).

Sectioned material

For electron microscopic observations of virus-induced

cytoplasmic inclusions, $2mm^2$ sections of leaf tissue were cut from Caladium hortulanum 'Candidum' and Dieffenbachia picta 'Exotica' leaves with symptoms. The leaf sections were placed in a 6% solution of glutaraldehyde in cacodylate buffer at pH 7.2. The tissue was placed under vacuum for 5 minutes and fixed for 1-3 hours at 4-5 C. The tissue was then washed twice for 15 minutes each with cacodylate buffer and post fixed for 1.5 hours at 23 C with 2% osmium tetroxide. The tissue was washed twice for ten minutes each with buffer and deionized water, respectively. The tissues were progressively dehydrated in a series of increasing concentrations of ethanol to 100%. At 70% ethanol they were stained for 5-10 hours in 2% uranyl acetate at 3-4 C. The tissue was subsequently transferred through two changes of 100% acetone for 15 and 30 minutes, respectively, at 23 C. The embedding plastic was an Epon 812, Araldite "M" mixture as described by Mollenhauer (47). The embedding procedure was done in three steps of 30, 70 and 100% plastic contained in acetone for 1 hour each after which it was placed in a vacuum oven at 60 C to remove the acetone. The tissue was placed in a drying oven overnight at 60 C and further polymerized in an oven at 80 C for 1-2 days.

Tissues were sectioned with glass knives mounted on a Sorvall Model MT-2 ultramicrotome. They were transferred to acid-cleaned, uncoated specimen grids and stained for 15 minutes in 0.5% aqueous uranyl acetate, rinsed in water, and subsequently stained for 5 minutes in lead citrate (58). The

sections were then rinsed in deionized water and allowed to dry prior to examination with the electron microscope.

Scanning Electron Microscopy

Apical shoots of 'Candidum' caladium were prepared for examination with a Cambridge Mark II stereoscanning electron microscope operating with an accelerating voltage of 10-20 KV. Shoot tissue comprising the apical dome and 1-2 leaf primordia were dissected and mounted on a 12mm diameter aluminum specimen stub covered with a thin layer of silver base paint. The specimens were examined as fresh material rather than being coated with metal.

Light Microscopy

Epidermal strips were removed from the abaxial surfaces of DMV-infected and healthy leaves of caladium, dieffenbachia and Colocasia esculenta (taro) and prepared for examination with a light microscope according to the procedures described by Christie (13,14) for staining virus-induced inclusions. Epidermal strips were placed in 5% Triton X-100 for 5 minutes and subsequently stained for 10 minutes in calcomine orange and "Luxol" brilliant green. The strips were rinsed briefly in 95% ethanol and mounted in Euparal on a glass slide.

Purification of Virus and Virus-induced Cytoplasmic Inclusions

Attempts were made to purify virus particles and cytoplasmic inclusions from leaves of 'Candidum' caladium and 'Exotica' dieffenbachia with symptoms and from entire symp-

tomatic shoots of the latter. All DMV-infected plants were obtained from commercial propagators in Orange County. All purification procedures were conducted at 4 C and 500 g of tissue were used in each purification run.

The virus purification procedure was as described by Hiebert and McDonald (36) except that the final step involved three rather than one cycle of differential centrifugation.

The procedure used to purify the cytoplasmic inclusions induced by DMV was as described by Hiebert et al. (37) and modified by Hiebert and McDonald (36).

Serology

Attempts were made to obtain antiserum specific to DMV. Virus purified from 'Exotica' dieffenbachia leaves was emulsified (1:1, v/v) with Freund's incomplete adjuvant and injected intramuscularly into a rabbit. Only one injection was made. The blood was collected 26 days after injection, incubated at 37 C in a water bath, and allowed to clot. The serum fraction was collected, placed in glass ampules, and frozen. Antigens were prepared by triturating 2 g of symptomatic or healthy 'Candidum' caladium leaf tissue with a mortar and pestle in 2 ml of deionized water. The triturated sap was then expressed through cheesecloth and centrifuged at approximately 2600 g for 30 minutes. The supernatant was then discarded and the pellet resuspended in 1 ml of water. In this trial, the gels consisted of 0.8% Noble agar,

0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide in water (24).

The Ouchterlony agar double-diffusion technique was used in all serological studies. The respective antisera were placed in center wells and the antigens in peripheral wells. Test plates were incubated 1-4 days at 25 C and observed periodically. In all tests, normal serum and leaf extracts from healthy plants were used as controls.

In other trials, attempts were made to determine the serological relationships of DMV to other viruses of the potato Y virus group. These tests were conducted with antisera to pepper mottle virus, potato virus Y, tobacco etch virus and turnip mosaic virus, obtained from D. E. Purcifull (Department of Plant Pathology, University of Florida, Gainesville). Antigens of DMV were prepared by triturating 4 g of symptomatic or healthy leaf tissue of P. selloum with a mortar and pestle in 8 ml of water. The triturated sap was then expressed through cheesecloth and centrifuged at approximately 2600 *g* for 30 minutes. The supernatant was discarded and the pellet was resuspended in 8 ml of a 3% aqueous pyrrolidine solution. Antigens were either derived from P. selloum seedlings which had been inoculated 8 or 56 days previously with a DMV isolate originally from 'Candidum' caladiums or from pooled leaf tissue from P. selloum seedlings which had been inoculated 13 days previously with DMV isolates from various naturally infected caladium varieties 'Itocapus', 'John Peed', 'Dr. Groover', 'Avalon Rose', 'W. B. Halderman'

and 'Crimson Wave'. Antigens to the other PVY-type viruses were obtained from D. Batchelor (Department of Plant Pathology, University of Florida). These antigens were prepared by triturating 2 g of leaf tissue in 4 ml of water. The sap was then expressed through cheesecloth, pipetted into vials and freeze dried. When virus samples were needed, 4 ml/vial of a 3% pyrrolidine solution was added. The solution was stirred and allowed to stand for 30 minutes prior to use. The gels used in these tests consisted of 0.6% Noble agar, 0.05M Borate buffer at pH 8.2 and 0.02% sodium azide.

Distribution and Effects of Dasheen Mosaic Virus

Surveys

Surveys were conducted from 1969-1971 in an attempt to assess the prevalence of DMV in Florida. These surveys included foliage nurseries in Orange and Dade counties, caladium fields in Highlands and Orange counties, Xanthosoma sagittifolium (cocoyam) fields in Dade County, an aquarium plant nursery in Palm Beach County and experimental and ornamental plantings of taro at the University of Florida, Gainesville. In addition, aroid samples were solicited from areas outside continental United States, and assessed for DMV infection. All plants received were maintained in isolation and destroyed upon completion of tests.

Infections of DMV were assessed on the basis of symptoms expressed and recovery of virus, to at least 1 of 4 or more manually inoculated seedlings of P. selloum. Certain samples

were further checked by electron microscopic examinations of negatively stained leaf extracts.

Symptom Expression

Throughout the course of this study, various aroids infected with DMV were maintained in greenhouses and periodically examined for symptoms expressed.

In one experiment, 70 rooted cane pieces of 'Exotica' dieffenbachia were obtained from a foliage nursery in Orange County, Florida. All were cuttings of the same developmental stage when collected, and all except 4 exhibited foliar distortion and mosaic. Half of the plants were placed in a greenhouse where the temperature varied from 20-40 C, whereas the others were maintained in a different greenhouse at 24-30 C. The light intensities of both greenhouses were equivalent and plants in both greenhouses were routinely watered and fertilized. Each plant was observed routinely from April 20, 1971 to March 1, 1972 and the symptoms expressed on each leaf recorded. On July 19, half the plants in each greenhouse were transplanted from 4 to 6 inch pots and examined for any resultant effects on symptom expression.

In a similar experiment, 6 cocoyam plants rendered free of DMV through tissue culture were selected and manually inoculated with DMV. Thereafter, these plants were maintained for 5 months in a greenhouse at 20-40 C. Throughout this time, these plants were checked periodically for foliar symptoms.

Quantitative Effects

The effects of DMV on growth of caladium, dieffenbachia, P. selloum and Z. elliottiana were assessed after manual inoculation. All planting stock used was propagated from seed and maintained in isolation until inoculated. Plants of P. selloum and Z. elliottiana were inoculated as seedlings and an equal number of non-inoculated seedlings served as controls. Seedlings of caladium and dieffenbachia were maintained in isolation until they attained a size suitable for vegetative propagation. They were then vegetatively propagated and genotypically identical pairs were selected for comparisons between inoculated and non-inoculated plants.

Inoculated and non-inoculated plants were maintained in pots on greenhouse benches for observation. All data except for Z. elliottiana were recorded within a 12 month period. Results for Z. elliottiana were recorded 2.5 years after inoculation during which time the corms were harvested, stored, and replanted twice. Fresh corm weights, leaf areas (maximum length X maximum width) and petiole lengths were recorded for caladium and Z. elliottiana. Fresh shoot weights, leaf areas, and shoot heights were recorded for dieffenbachia and total fresh plant weights and leaf areas were recorded for P. selloum.

Elimination of Dasheen Mosaic Virus

Seed Propagation

Seed of caladium, dieffenbachia, and Zantedeschia spp.

were produced by intraspecific crosses. These crosses were made between plants of 'Candidum' caladium, 'Exotica' dieffenbachia, Z. albo-maculata, Z. elliottiana, or Z. rehmannii. All plants used in the crosses were shown to be infected with DMV based on symptoms and transmission to seedlings of P. selloum. Dieffenbachia plants were grown in a stock bed in a fiberglass house at Apopka and pollinated May-June, 1971. The caladiums were grown in Gainesville either in a greenhouse or outdoors and pollinated May-June, 1972. The Zantedeschia spp. were grown in a greenhouse at Gainesville maintained at 24-30 C and pollinated May-June, 1971. The pollination procedures were similar to those described by McColley and Miller (46) for philodendron. All crosses were made between 6-10 a.m. or 5-8 p.m. Pollen was collected daily with a camel-hair brush and transferred within 48 hours after shedding to neighboring receptive blooms of different plants. Bloom receptivity was indicated by the increased stickiness of the stigmatic surfaces of the spadix. Bloom receptivity of caladium and dieffenbachia was further indicated when the spathe began to unfurl revealing the distal portion of the spadix. Prior to pollination, the spathe was cut away from the spadix, discarded, and the pollen was gently applied to the proximal ovulate portion of the spadix with a brush.

Shoot-tip Culture

Attempts were made to culture the following aroids in vitro: 'Candidum' and 'Frieda Hemple' caladium, taro, cocoyam, 'Exotica' dieffenbachia, Aglaonema modestum (chinese evergreen),

P. selloum, Cryptocoryne cordata and C. nevillii.

In this study, the "revised" medium of Murashige and Skoog (50) was used except that (i) meso-inositol (i-inositol dihydrate) was substituted for myo-inositol, (ii) Edamin was omitted and (iii) each liter of medium contained 8 rather than 10 g of agar. The kinetin level employed was 1.0 mg/l of medium and the indole-acetic acid level was 15.0 mg/l.

Shoot-tips from all plants were excised and treated sequentially as follows: shoots were (i) trimmed to ≤ 1.5 cm^3, (ii) rinsed in flowing tap water, (iii) submerged for 10 minutes in 0.52% sodium hypochlorite, (iv) trimmed further, (v) submerged for 5 minutes in 0.26% sodium hypochlorite and (vi) rinsed briefly in sterile distilled water. With the aid of a dissecting microscope, each shoot was then trimmed until only the apical dome and one or two leaf primordia remained (Fig. 13A,B). Finally, each shoot was transferred, cut surface down, to the surface of 15 ml of solidified medium contained in a 30 ml screw-cap vial. All cultures were maintained at 21-25 C and provided 12 hours of daily light (ca. 750 ft-c) from incandescent and fluorescent bulbs.

Caladium, cocoyam and taro plantlets which had differentiated in culture were separated from undifferentiated callus and transplanted individually to sterilized sand/peat (1:1) contained in 7.5 cm clay pots. All potted plantlets were placed in insect-proof cages in a growth room and observed for virus symptoms before being transplanted to larger pots and transferred to greenhouses. Differentiated Cryptocoryne

spp. plantlets were transferred to sterilized sand contained in 3.8 l wide-mouth glass jars.

Special precautions were taken to ascertain the presence or absence of infected material. Plants were maintained for at least 3 months in observation cages before being transferred to greenhouses. Samples were further checked for DMV infection by electron microscopic examination of leaf dip preparations and/or through manual transmission attempts to seedlings of P. selloum.

RESULTS

Characterization of Dasheen Mosaic Virus

Host-range Determinations

Dasheen mosaic virus proved to have a wide host range within the Araceae and was recovered from members of 9 of the 12 tribes and 13 of the 16 genera represented (Table 1). The following aroids did not become infected when manually inoculated as seedlings: Aglaonema commutatum, Anthurium bakerii, A. crispimarginatum, Peltandra sp., P. stratiotes and Spathiphyllum floribundum. None of the non-araceous plant species including Tetragonia expansa proved susceptible to DMV when manually inoculated (Table 2).

Neither host range differences nor differences in symptom expression were noted when single isolates from 'Candidum' caladium, 'Exotica' dieffenbachia and taro were compared. All three isolates infected manually inoculated seedlings of Anthurium scandens, caladium, dieffenbachia and P. selloum, and all three isolates induced identical symptoms in P. selloum seedlings inoculated in recovery trials.

Table 1. Araceous plants susceptible to dasheen mosaic virus[a].

Tribe[b]	Species	Cultivar	DMV Infection[c]
Aglaonemateae	Aglaonema modestum		A
Amorphophalleae	Amorphophallus campestrus		A
			A
Anthurieae	Anthurium aff. loefgrenii		B
	A. scandens		B
Areae	Arisaema triphyllum		B
	Cryptocoryne cordata		A
Colocasieae	Alocasia sp.		A
	Caladium hortulanum	Avalon Rose	A
		Candidum	A,C
		Caroline Whorton	A
		Crimson Wave	A
		Dr. Groover	A
		Exposition	A
		Frieda Hemple	A,C
		Itacapus	A
		John Peed	A
		Lord Derby	A
		Miss Chicago	A
		Red Ensign	A
		W. B. Halderman	A

Table 1 continued

Tribe[b]	Species	Cultivar	DMV Infection[c]
		White Christmas	A
		Unnamed hybrids[d]	B
	Colocasia esculenta		A,C
	Unidentified Colocasia sp.		A
	Xanthosoma brasiliense		A
	X. sagittifolium	Malanga Blanca	A,C
Dieffenbachia	Dieffenbachia picta	Exotica	A
		Perfection	A
		Unnamed hybrids[d]	B
	D. amoena		A
Philodendreae	Philodendron pittieri		A
	P. selloum		A,B
Spathiphylleae	Spathiphyllum kochii		B
Zantedeschieae	Zantedeschia aethiopica		A
	Z. albo-maculata		A,B
	Z. elliottiana		A,B
	Z. rehmannii		A,B

[a] For all plants listed, DMV was either recovered to P. selloum seedlings from naturally infected plants or proved susceptible when manually inoculated as seedlings. In every

Table 1 continued

instance, susceptibility to DMV was judged on the basis of typical symptoms induced in P. selloum and the presence of filamentous virus particles observed in leaf extracts.

[b] Tribal classification is given as listed by Engler (21).

[c] A = DMV recovered from naturally infected plants, B = manually inoculated with DMV as seedlings, C = plants derived from shoot-tip cultures and manually inoculated with DMV.

[d] Plants grown from seed.

Table 2. Plants not infected with dasheen mosaic virus following manual inoculations[a]

Family	Species	Cultivar
Aizoaceae	Tetragonia expansa	
Amaranthaceae	Amaranthus tricolor	
	Gomphrena globosa	
Amaryllidaceae	Amaryllis sp.	
Bromeliaceae	Aechmea pubescens	
Chenopodiaceae	Beta vulgaris	
	Chenopodium amaranticolor	
	Chenopodium quinoa	
Commeliniaceae	Rhoeo discolor	
Compositae	Zinnia elegans	
Convolvulaceae	Ipomoea purpurea	Scarlet
Cruciferae	Brassica perviridis	Tender green
Curcurbitaceae	Cucurbita pepo	Small sugar
	Curcurbita pepo var. melopepo	Early Prolific Straightneck
Gramineae	Avena sativa	Red Rustproof
	Lolium sp.	Ryegrass
	Pennisetum typhoideum	Pearl
	Sorghum vulgare	Georgia 615
	Triticum aestivum	Michigan Amber
	Zea mays	Golden Cross Bantam

Table 2 continued

Family	Species	Cultivar
Iridaceae	Gladiolus hortulanus	
Labiatae	Ocimum basilicum	
Leguminosae	Arachis hypogaea	Florunner Early Runner Dixie Runner
	Cassia occidentalis	
	Cassia tora	
	Cyamopsis tetragonoloba	
	Desmodium canum	
	Glycine max	Bragg
	Phaseolus vulgaris	Red Kidney Michelite 62
	Pisum sativum	Alaska Little Marvel
	Vicia faba	Longpod
	Vigna unguiculata	Early Ramshorn Blackeye
Liliaceae	Allium cepa	Yellow Bermuda
	Lilium longiflorum	
Phytolaccaceae	Phytolacca americana	
Solanaceae	Capsicum frutescens	California Wonder
	Datura stramonium	
	Lycopersicon esculentum	Manalucie
	Nicotiana clevelandii	
	Nicotiana glutinosa	

Table 2 continued

Family	Species	Cultivar
	Nicotiana hybrid[b]	
	Nicotiana sylvestris	
	Nicotiana tabacum	Samsun NN
	Petunia hybrida	
	Physalis floridana	

[a] All plants tested were inoculated as seedlings. In every instance attempts were made to recover DMV to P. selloum 2-6 weeks after inoculation.

[b] Nicotiana hybrid (N. clevelandii x N. glutinosa) developed by S. R. Christie (15).

Transmission Electron Microscopy

Leaf extracts

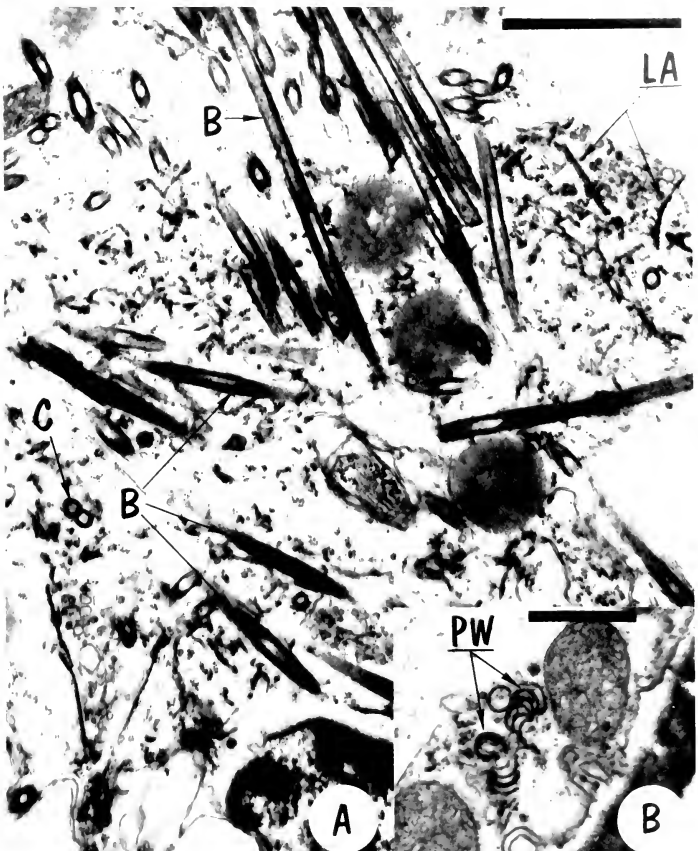
Electron microscopic examinations of negatively stained leaf dip preparations of various aroids exhibiting mosaic symptoms revealed the presence of filamentous rods (Fig. 3B) similar to those reported by Zettler et al. (74,75). Measurements of virus particles from cocoyam plants infected with a DMV isolate originally from 'Candidum' caladium were similar to those previously reported for DMV in taro (74,75); i.e., 77% of 95 particles measured were 726 to 815 nm long with a main maximum at 801 nm and a mean length of 782 nm. Such particles were not seen in extracts from healthy cocoyam plants.

Striated inclusions were seen in leaf extracts of 'Candidum' caladium and 'Exotica' dieffenbachia leaves stained with ammonium molybdate. These inclusions resembled those reported by others for viruses of the potato Y virus group (20). Tubes and laminated aggregates were observed in leaf extracts from both caladium and dieffenbachia. No apparent differences in specific inclusion morphology were noted between these species.

Sectioned material

Ultrathin sections of 'Candidum' caladium infected with DMV revealed the presence of pinwheel, circle, bundle and laminated aggregate inclusions (Fig. 2) similar to those described by Zettler et al. (74,75) for DMV-infected taro plants. Of the sections examined, the relative numbers of tubes seemed to be much greater than the number of pinwheels or laminated aggregates. Virus particles were commonly found in close association

Figure 2. A,B) Thin sections of a caladium leaf infected with dasheen mosaic virus showing pinwheel (PW), circle (C), bundle (B) and laminated aggregate (LA) inclusions. Scale line of A is 900 nm and B is 450 nm. Note close association of virus particles with inclusions.


LA
B
C
B
A
PW
B

with these cytoplasmic inclusions (Fig. 2).

Light Microscopy

Amorphous cytoplasmic inclusions were seen in stained epidermal strips taken from 'Candidum' caladium, 'Exotica' dieffenbachia and taro when examined with a light microscope (Fig. 3). Neither nuclear nor nucleolar inclusions were observed in any of the epidermal strips. No amorphous cytoplasmic inclusions were observed in epidermal strips from healthy plants of caladium, dieffenbachia and taro.

Purification of Virus and Virus-induced Cytoplasmic Inclusions

DMV particles were partially purified from dieffenbachia shoot and leaf tissue (Fig. 5C). This material had a maximum ultraviolet absorbtion at 260 nm in aqueous 0.02M Tris buffer, pH 7.4, and 10^{-3}M Cleland's reagent. The UV absorption spectrum from 230-360 nm was very similar to that reported for other PVY type viruses (Fig. 4). The 260/280 ratio of ca. 1.507 (not corrected for light scattering), however, is a value higher then that recorded for other flexous-rod shaped viruses (personal communication, E. Hiebert).

Attempts to purify particles of DMV from caladium were unsuccessful. A green mucilaginous pellet resulted from the first high speed centrifugation. The purification procedures were discontinued at this step since the pellet proved difficult to resuspend and contained a relatively low concentration of virus particles based upon electron microscopic examination.

DMV-induced cytoplasmic inclusions were partially purified

Figure 3. A) Stained epidermal cells of caladium leaf infected with dasheen mosaic virus. Note amorphous cytoplasmic inclusions (arrows). B) Negatively stained particles of dasheen mosaic virus from caladium leaf extract. Scale line is 400 nm.

A
B

Figure 4. Ultraviolet absorption spectrum (not corrected for light scattering) of dasheen mosaic virus, purified from 'Exotica' dieffenbachia and contained in 0.02 M Tris buffer and 10^{-2}M Cleland's reagent.

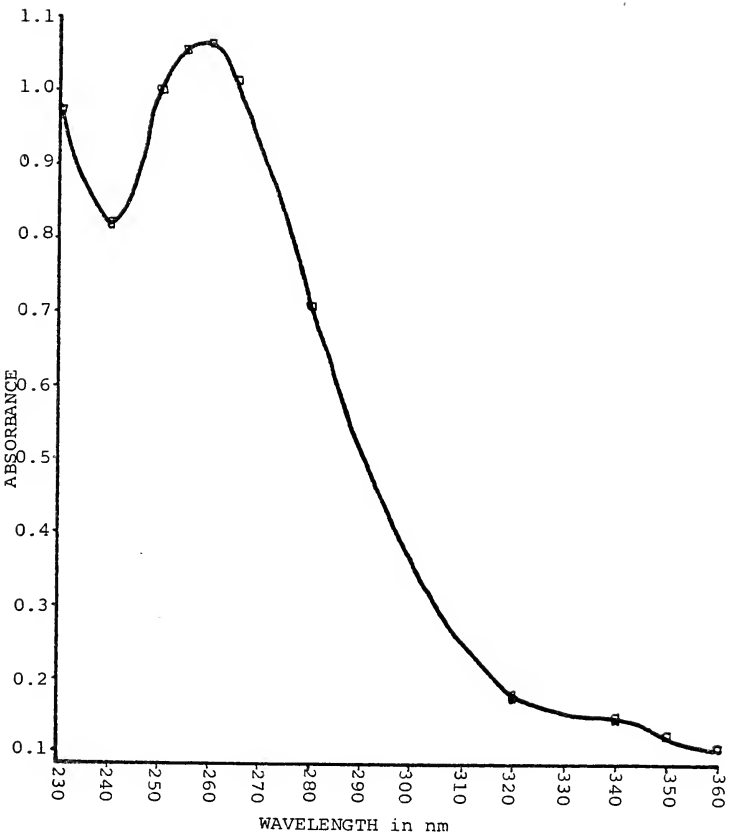
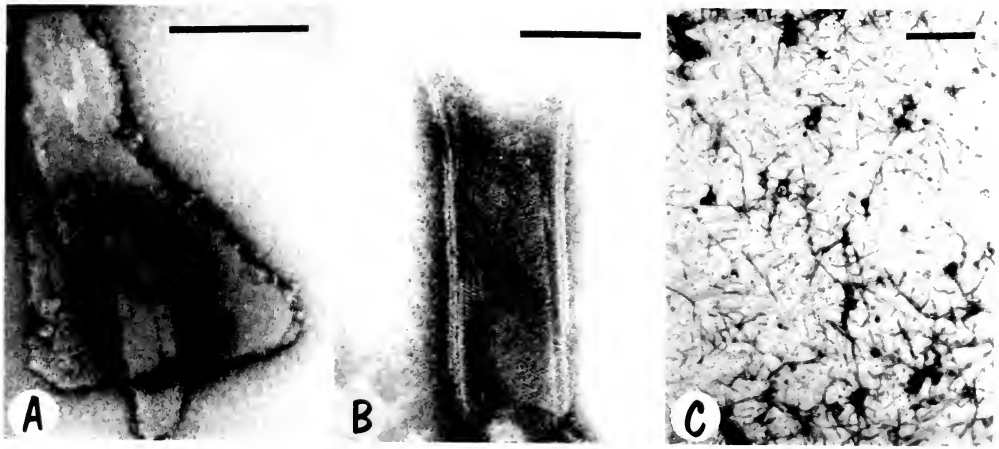

1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
ABSORBANCE
230
240
250
260
270
280
290
300
310
320
330
340
350
360
WAVELENGTH in nm

Figure 5. A) Laminated aggregate inclusion purified from 'Exotica' dieffenbachia leaf tissue infected with dasheen mosaic virus. Note striations. Scale line is 300 nm. B) Tubular inclusion purified from 'Candidum' caladium leaf tissue infected with dasheen mosaic virus. Note striations. Scale line is 150 nm. C) Particles of dasheen mosaic virus partially purified from 'Exotica' dieffenbachia leaf tissue. Scale line is 800 nm.


A
B
C

from symptomatic caladium and dieffenbachia leaves and dieffenbachia shoots. Striated plate-like inclusions as well as striated tubular inclusions were observed from both dieffenbachia and caladium tissue. Whereas plates predominated in dieffenbachia extracts (Fig. 5A), tubes predominated in extracts of caladium (Fig. 5B).

Serology

No reactions were noted between center wells containing serum prepared from DMV and peripheral wells with extracts from healthy or DMV-infected caladium leaves.

Precipitin reactions formed between peripheral wells with extracts of DMV-infected P. selloum leaves and center wells containing antisera to TEV and PVY (Fig. 6). Nevertheless, the homologous reactions were much stronger and spurred over the heterologous reactions. The heterologous reactions were stronger when DMV antigens were prepared from P. selloum plants inoculated 10-13 days prior to extraction than from plants inoculated 56 days earlier.

No reactions were observed between antisera of pepper mottle or turnip mosaic viruses and extracts from DMV-infected P. selloum leaves. Nor were any reactions observed between any of the antisera wells and peripheral wells containing extracts of healthy tobacco or P. selloum leaves.

Figure 6. Serological relationship of dasheen mosaic virus (DMV) to potato Y (PVY) and tobacco etch (TEV) viruses. The center wells contain: (A) PVY antiserum, (B) TEV antiserum, (C) Normal serum. The peripheral wells in each pattern were filled with pyrrolidine treated tissue extracts from: (1) healthy tobacco, (2) PVY-infected tobacco, (3) and (5) DMV-infected Philodendron selloum, (4) TEV-infected tobacco, and (6) healthy P. selloum.


1
6
2
A
5
3
4
1
6
2
B
5
3
4
1
6
2
C
5
3
4

Distribution and Effects of Dasheen Mosaic Virus

Surveys

Symptoms of DMV infection were observed in all commercial, ornamental and experimental aroid plantings surveyed in Florida. Areas surveyed included: 4 and 1 caladium fields in Highlands and Orange counties, respectively, 4 cocoyam fields in Dade County, 1 experimental and 2 ornamental taro plantings in Alachua County, 1 aquarium plant nursery in Palm Beach County, and 3 and 6 foliage nurseries in Dade and Orange counties, respectively. Dasheen mosaic virus was recovered to seedlings of P. selloum, and filamentous particles were observed in leaf dip preparations from the following aroids obtained in these surveys: A. rodestum, C. cordata, caladium ('Candidum', 'Caroline Whorton', 'Crimson Wave', 'Dr. Groover', 'Exposition, 'Frieda Hemple', 'Miss Chicago', 'White Christmas'), taro, an ornamental Colocasia sp., Dieffenbachia amoena and dieffenbachia ('Exotica', 'Perfection') and cocoyam.

Dasheen mosaic virus was also recovered to P. selloum and virus particles observed in taro samples received from India, Hawaii, and the Fiji Islands, and in cocoyam samples from Trinidad. A sample of Xanthosoma brasilensis from Guadeloupe likewise proved infected with DMV.

Symptom Expression

Foliar mosaic symptoms were observed in all plants from which DMV was recovered (Table 1). Dasheen mosaic virus, however, was rarely isolated from tissues without such symptoms.

The foliar mosaic pattern of taro and cocoyam was typically associated with the major veins and resembled a "feathery" appearance (Fig. 8B), a characteristic noted by others (2,17, 22,25). A "vein mosaic" pattern (6) was typically expressed on the first leaves of P. selloum seedlings to show symptoms after inoculation with DMV (Fig. 7C). On subsequently formed leaves, however, this veinal pattern was not apparent (Fig. 7D).

Prominent foliar distortion symptoms were frequently noted for certain aroids, including 'Exotica' and 'Perfection' dieffenbachia (Fig. 7A), Philodendron pittieri, P. selloum, Zantedeschia aethiopica, Z. albo-maculata and Z. elliottiana. Distortion symptoms induced by DMV in dieffenbachia included epinasty and, in many instances, a marked disruption of the vertical symmetry of the unfurled leaf (Fig. 7A). As such leaves became fully expanded, torn regions resulted due to uneven expansion rates of maturing lamellar tissues.

Whereas DMV symptoms of some aroids were not conspicuous and did not markedly deter from their attractiveness, other aroids were severely affected, particularly 'Exotica' and 'Perfection' dieffenbachia and Z. elliottiana. Among variegated aroids such as caladium and dieffenbachia, DMV-induced mosaic symptoms supplanted inherited patterns, which detracted from their attractiveness (Fig. 7B, 8A).

Several authors have observed that leaves with symptoms appear intermittently among infected plants (2,75). This phenomenon was apparent for most aroids observed in this study,

Figure 7. A) Epinastic response to dasheen mosaic virus by dieffenbachia plants inoculated 10 days previously. B) DMV symptoms of 'Exotica' dieffenbachia, leaves with symptoms (right) and leaf without symptoms (left). Note size reduction and impairment of variegation in leaves with symptoms. C) Leaves of healthy (right) and diseased (left) seedlings of Philodendron selloum. Leaf on left is from a plant inoculated 10 days previously with dasheen mosaic virus. D) Mosaic symptoms on leaf of P. selloum typical of plants inoculated 2-3 months previously with dasheen mosaic virus.

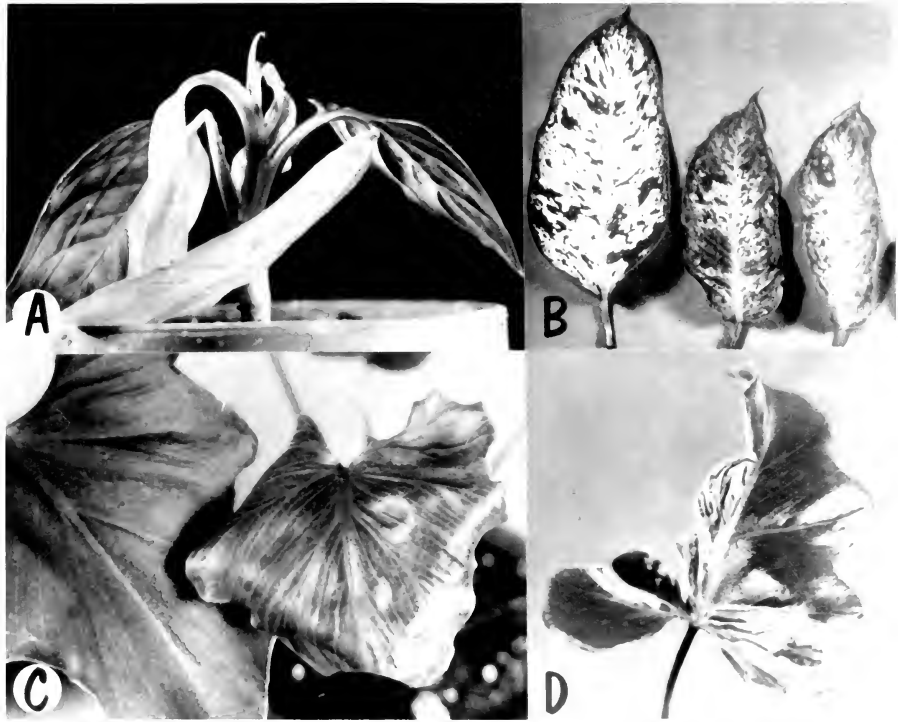
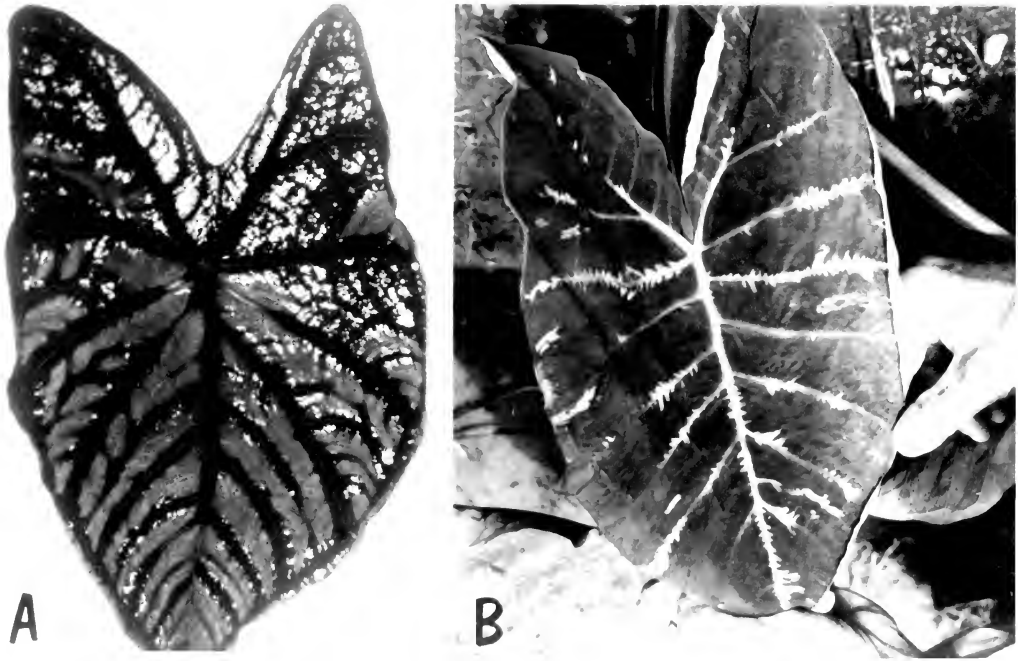

A
B
C
D

Figure 8. A) Mosaic symptoms induced by dasheen mosaic virus in leaf of 'Candidum' caladium. Note impairment of normal pattern of variegation. B) Feathery foliar mosaic symptoms induced by dasheen mosaic virus in cocoyam. Such patterns are frequently associated with the major leaf veins.


A
B

including caladium, cocoyam, dieffenbachia, P. selloum and taro. Infected plants of certain species, however, such as P. pittieri expressed symptoms continually throughout the observation period.

In one experiment involving cocoyam plants inoculated with DMV, successively produced leaves were examined for expressed symptoms (Table 3). For all six plants, mosaic symptoms were apparent on the first leaf to appear following inoculation. Thereafter, leaves with and without symptoms were produced on each of the plants, and, with one exception, leaves with symptoms appeared in succession following the emergence of symptomless leaves. The duration of this intermittent expression of symptoms among aroids was not assessed in this study, although it was noted that plants of P. selloum inoculated with DMV as seedlings in October, 1969, still intermittently produced leaves with and without mosaic symptoms 4.5 years later.

An unsuccessful attempt was made during this investigation to correlate certain environmental conditions with the appearance of symptoms using DMV-infected 'Exotica' dieffenbachia plants. Both temperature and pot size, as it relates to growth rate, were studied. The periodicity of symptom expression was identical at both temperature regimes tested, although plants growing in the greenhouse where temperature fluctuated between 20 and 40 C averaged 16.2 leaves per plant compared to only 12.6 leaves per plant for the plants growing where the temperature was controlled between 24-30 C (537 and 429 leaves per 33 and 34 plants, respectively). At both

Table 3. Succession of leaves emerging from cocoyam plants and rated according to symptoms expressed[a] following inoculation with dasheen mosaic virus; study period August, 1973-January, 1974

Plant No.[b]	Leaf Number					
	1	2	3	4	5	6
1	+	+	-	-	+	-
2	+	-	+	+	+	-
3	+	+	+	-	+	-
4	+	-	-	+	+	+
5	+	+	+	+	+	-
6	+	-	-	-	+	+
% with symptoms	100	50	50	50	100	33

[a] + = leaf with mosaic symptoms, - = without symptoms.

[b] Plants used in this study were, prior to inoculation, virus-free and originated from a single shoot-tip cultured in vitro.

regimes, the lowest proportion of leaves with symptoms emerged June-September whereas leaves with the highest proportions emerged October-December and April-May (Fig. 9). This symptom periodicity data generally correspond with observations of 'Exotica' dieffenbachia plants grown commercially at Apopka, Florida (Knauss, personal communication). Similarly, no effect on symptom expression was noted in this experiment as a result of transplanting plants from 4 to 6" pots, although plants transferred to larger pots grew more vigorously than their counterparts in smaller containers: at 20-40 C and 24-30 C, an average of 16.8 and 13.2 leaves per plant were produced in 6" pots compared to only 15.6 and 11.9 leaves per plant in 4" pots, respectively (303 and 238 leaves for 18 and 18 plants and 234 and 191 leaves for 15 and 16 plants, respectively).

Quantitative Effects

Plants infected with DMV proved stunted in comparison to their healthy counterparts (Table 4) regardless of whether or not mosaic symptoms were evident on plants when recorded. Fresh corm weights and leaf areas (maximum length x maximum width) of 69 caladium plants were 40 and 53% less than healthy plants, respectively. Corm weights and leaf areas of 8 Z. elliottiana plants were likewise reduced 59 and 51%, respectively. Similarly, fresh shoot weights and leaf areas of 27 infected dieffenbachia plants were 63 and 66% less than controls; and total fresh plant weights and leaf areas of 36 P. selloum plants were 30 and 30% less, respectively (Table 4).

Figure 9. Symptoms of dasheen mosaic virus expressed in newly emerged leaves of 'Exotica' dieffenbachia plants in relation to date of emergence and temperature regime at which plants were maintained. Thirty-four and 33 plants were maintained at 24-30 and 20-40 C, respectively. Bars indicate the percentage of leaves with symptoms according to the order of foliar succession.

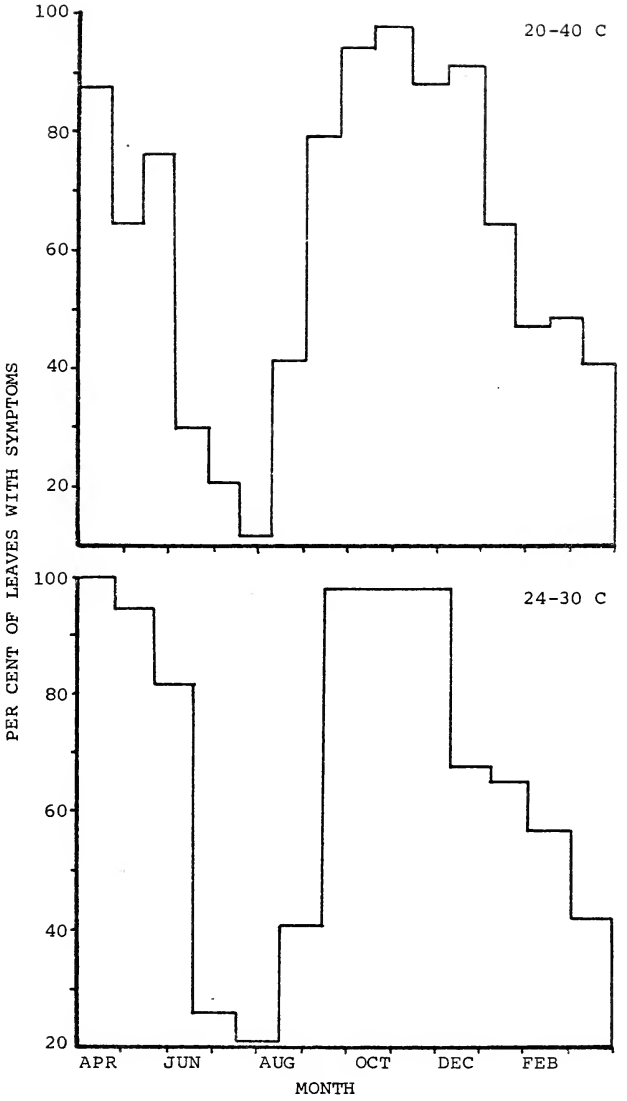

20-40 C
24-30 C
PER CENT OF LEAVES WITH SYMPTOMS
100
80
60
40
20
APR
JUN
AUG
OCT
DEC
FEB
MONTH

Table 4. Effects of dasheen mosaic virus on fresh weights and leaf dimensions of plants of caladium, dieffenbachia, Philodendron selloum and Zantedeschia elliottiana[a,b,c]

Plant	Weights (g)[e]			Leaf Dimensions (cm)[d]								
				Width			Length			Petiole Length		
	I[f]	H	%	I	H	%	I	H	%	I	H	%
Caladium	12.9	21.5	40	10.2	14.6	33	12.7	18.8	32	18.7	31.8	41
Dieffenbachia	12.9	35.4	63	3.9	6.5	43	8.7	15.2	40			
P. selloum	153.2	219.3	30	18.2	22.2	18	16.3	19.1	16			
Z. elliottiana	15.7	38.1	59	16.2	22.3	27	22.4	33.0	32	32.3	49.3	35

[a] All plants tested were propagated from seed and maintained in isolation until inoculated.

[b] Results recorded 4,5,9 and 30 months after inoculation, respectively.

[c] Sixty-nine, 27, 36 and 8 plants inoculated, respectively, and compared with equal numbers of controls. All data represent average values.

[d] Data based upon largest leaf of each plant when recorded.

[e] Data for caladium and Z. elliottiana based upon corm weights; data for dieffenbachia and P. selloum based upon shoot and total plant weights, respectively.

[f] I = infected, H = healthy and % = percent reduction due to virus.

All these results were highly significant at the 1% level.

Elimination of Dasheen Mosaic Virus

Seed Propagation

Dieffenbachia fruits, although cream colored during most of their development, became red when ripe approximately 10-12 weeks after pollination. Dieffenbachia fruits did not dehisce but remained loosely attached to the spadix (Fig. 10). A spadix bore 15-30 ovaries, each containing a single round seed 5-6 mm in diameter which was green when mature. Seeds germinated within 20 days after they were removed from the fruit and planted in moist peat.

Unlike dieffenbachia, caladium fruits ripened 5-6 weeks after pollination and abscissed from the spadix (Fig. 11). The exposed fruit surfaces remained green throughout their development without apparent color change at the time of abscission. The exposed surfaces of the ovary walls were cream-colored at maturity. Ovulate portions of the spadix contained approximately 200 seed-bearing ovaries, each containing 1-14 oval seeds which were 1-1.5 mm in length and light tan in color. As many as 1500 seeds were obtained from a single spadix. Seeds were removed from the fruit and planted in moist peat. Seeds germinated readily 8-10 days after planting. Seed germination rate decreased markedly, however, when seeds were dried and stored 2 weeks at 23 C and 53% relative humidity.

Fruits of Zantedeschia spp. also remained green throughout

Figure 10. Dieffenbachia spadix. A) during pollen shed. B) developing fruit 5-6 weeks after pollination. C) mature fruit 10-12 weeks after pollination.

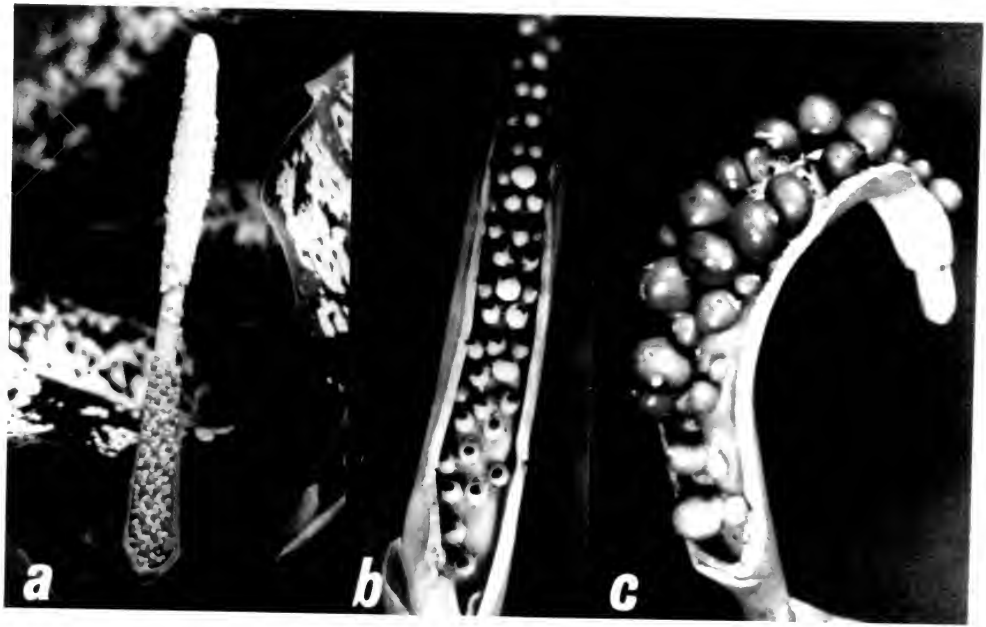

a
b
c

Figure 11. Caladium spadix. A) at time of pollination. B) distal portion during pollen shed. C) proximal portion just prior to fruit abscission. D) shed fruit at abscission 5-6 weeks after pollination.

a
b
c
d

their development, but did not abscise from the spadix at maturity (4-6 weeks after pollination). Each fruit contained one seed.

Seedlings were maintained in a greenhouse at about 60% shade in isolation from commercial aroid stock. Plants were transferred from peat to a steam-sterilized soil/perlite mix contained in clay pots and treated routinely with a liquid formulation of 20-20-20 fertilizer.

Although parental 'Exotica' dieffenbachia, 'Candidum' caladium, and Zantedeschia spp., plants used in this study were virus-infected as evidenced by symptoms observed at the time of pollination, none of the more than 500 resulting dieffenbachia, 1000 caladium and 100 Zantedeschia progeny displayed virus symptoms, indicating that the seedlings were virus-free.

A small percentage of the dieffenbachia seedlings exhibited albinistic tendencies and died shortly after germination. The juvenile foliage of the surviving dieffenbachia progeny was non-variegated and variegated leaves did not develop until 4-8 months after germination. Similarly, juvenile leaves of caladium were homogeneously green and variegated leaves did not develop until about 3 months after germination.

A year after germination a marked variation was noted among the 207 randomly selected dieffenbachia seedlings which had grown to 30-34 cm in height. Fifty-three of the progeny exhibited none of the white variegation typical of the 'Exotica' parents. The foliar patterns of the remaining progeny varied from those having relatively localized areas of white to those

having white patterns exceeding in area that of the original parents (Fig. 12A). A few plants exhibited a degree of foliar coloration unlike the 'Exotica' parents. Marked differences in leaf shape and apical dominance were also noted among the progeny. Whereas some of the leaves had dimensions similar to those of the parents, others were somewhat more lanceolate. Axillary buds of some progeny grew only after the apical meristem was removed whereas the axillary buds of other progeny proliferated regardless of the presence or absence of the apical shoot (Fig. 12B).

Little variation was noted among the 100 randomly selected caladium seedlings 8 months after germination. Fifty-one percent of the progeny had predominately white leaves with 22 and 27% having predominately green or intermediately white leaves, respectively. Forty-one percent had striped petioles with 55 and 4% having predominately green or purple petioles, respectively. The parent 'Candidum' plants by comparison have predominately white leaves with striped petioles. Minor differences were noted in leaf shape or apical dominance between the 'Candidum' parents and the progeny.

All seedlings of Zantedeschia spp. resembled the parents from which they were derived. These data correspond closely to those obtained by Ryohitsu (60) for the self-pollinated progeny of yellow, white and pink Zantedeschia spp.

Shoot-tip Culture

Attempts to culture pathogen-free plants of caladium, taro and cocoyam were apparently successful. Forty of 50,

Figure 12. Dieffenbachia seedlings 12 months after germination. Note differences in foliar variegation patterns (A) and in degree of shoot proliferation (B).

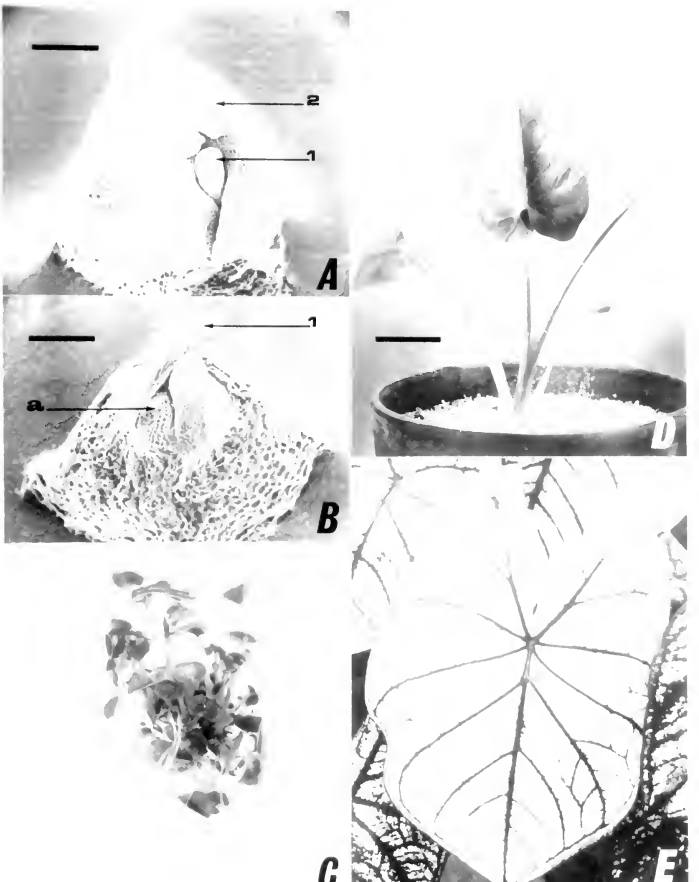

A
B

3 of 6, 4 of 6 and 6 of 6 shoots of 'Candidum' and 'Frieda Hemple' caladium, taro and cocoyam, respectively, developed *in vitro* without apparent microbial contamination. Shoot tips, originally cream colored, became green within 2 weeks after excision. Within 8 weeks, callus masses developed and differentiated numerous shoot buds. Within 18 weeks, many plantlets with roots and shoots developed from these buds (Fig. 13C). Plantlets of all three species survived transfer to soil and developed rapidly into vigorously growing plants (Fig. 13D).

Every 3 months each caladium culture generated 10-20 plantlets suitable for transplanting and enough callus for an additional 10-20 cultures. 'Candidum' caladium callus has been maintained in culture for at least 1.5 years without apparent adverse affects expressed by plantlets after transfer to soil.

All attempts to culture dieffenbachia, chinese evergreen and philodendron were unsuccessful either because microbe-free shoot tips could not be obtained or because they failed to grow *in vitro*. Six of 60, 2 of 2 and 2 of 3 shoot-tips of dieffenbachia, chinese evergreen and philodendron, respectively, were without apparent microbial contamination. However, none of these developed callus or differentiated into plantlets. Shoot-tips, originally cream colored, became green 1-2 weeks after excision, but eventually became necrotic and died. Similar attempts with *C. cordata* and *C. nevillii* were successful; however, both of these species failed to proliferate in culture.

Figure 13. (A,B) Scanning electron micrograph of caladium apical shoot showing first (1) and second (2) leaf primordia and apical dome (a). Scale line is 0.166 mm. C) Six-week-old culture of 'Frieda Hemple' caladiums on Murashige-Skoog medium. D) Cocoyam plant 6 weeks after transfer to soil from culture. Scale line is 5.4 cm. E) Mature pathogen-free 'Candidum' caladiums grown under greenhouse conditions.


2
1
A
1
a
B
C
D
E

Three of 10 and 2 of 5 shoot tips of C. cordata and C. nevillii, respectively, were without microbial contamination and became green 1-2 weeks after excision but differentiated only one plantlet per shoot tip.

Attempts to culture dieffenbachia on other media were likewise unsuccessful. Three of 20 and 2 of 20 apical shoots proved microbe free when placed on Vacin-Went (68) and Knudson C (43) medium, respectively. However, as with the Murashige-Skoog medium, none of the 5 shoots survived in culture.

Plantlets of 'Candidum' caladium and taro obtained from some shoot tips in culture proved to be infected with DMV. Virus-infected plantlets were readily detected in vitro and as plants growing in soil. These observations were confirmed through inoculations of P. selloum seedlings and electron microscopy. Plants of both caladium cultivars, taro and cocoyam derived from other shoot-tips, however, proved free of virus and were used in continued propagations.

Virus-free plants of 'Candidum' and 'Frieda Hemple' caladiums developed typically variegated leaves 10-12 weeks after transplanting into soil. Some of the 'Candidum' caladiums flowered 6-7 months after transplanting and produced corms 4-5 cm in diameter with leaves 20-27 cm wide and 25-32 cm long (measured from lamella tip to point of petiole attachment) (Fig. 13E). After 1 year, these plants had corms ca. 9.5 cm in diameter with a fresh harvest weight of ca. 200 g. Mature plants were identical to their commercial counterparts, except that they appeared to be more vigorous. In one experiment,

14 DMV-free and 14 DMV-infected plantlets were transplanted from culture to soil and maintained under identical growing conditions. After 6 months, corm weights of healthy plants were significantly greater (t value = 2.6; P = < 5%) than those from diseased plants (56.9 and 40.2 g/corm, respectively).

Approximately 350 virus-free 'Candidum' caladium plantlets have been grown to maturity since this study was initiated. These plants were propagated in a screened greenhouse on raised asbestos-cement benches previously sterilized with a 5% commercial formulation of sodium hypochlorite. The plants were routinely treated with either nicotine sulfate or malathion for insect control and twice with Truban (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole) for the control of pythiaceous fungi. During their development, none of the plants ever had symptoms of DMV. At harvest (ca. 6 months after planting), none of the plants had apparent discolored or necrotic roots or corms.

DISCUSSION

This study provides additional evidence that DMV is a member of the potato Y group of viruses as originally described by Brandes (7), supplemented by Edwardson (18), and recognized by the International Committee on Nomenclature of Viruses (25, 72). The mean length of 782 nm for particles in leaf extracts from DMV-infected cocoyam plants corresponds closely to the values of 759 nm for particles in cocoyam by Debrot and Ordosgoitti (17) and 750 and 751 nm for particles in taro by Zettler et al. (74,75) and Debrot and Ordosgoitti (17), respectively. Similar flexous rod particles were found in leaf extracts of DMV-infected plants representing 12 other aroid genera investigated in this study. Similarly, cylindrical inclusions as described by Edwardson et al. (20) were observed in extracts and/or thin sections of leaves from DMV-infected plants of caladium, dieffenbachia and Z. elliottiana. These inclusions closely resembled those described by Zettler et al. (74,75) for DMV-infected taro except that, in dieffenbachia, laminated aggregate inclusions were more prevalent than tubular inclusions.

The relatedness of DMV to potato Y and tobacco etch viruses as indicated by serological data further supports the contention that DMV is a member of this virus group. These serological

results were confirmed by other workers who found that DMV and 7 other viruses considered to be members of this group reacted positively to antiserum of tobacco etch D-protein (62). Attempts to obtain an antiserum specific to DMV were not successful in this study. This is presumably due to relatively low virus titers in the leaf tissues used for purification and to a relatively low purity of the final injected viral preparation, as indicated by the relatively high 260/280 value of 1.59 recorded.

Herold (34,35) reported a mean particle length of 754 nm for a virus of Anthurium andraeanum which was reported to be transmitted by white flies and manually transmissible from tobacco to other solanaceous plant species. Although DMV was recovered from and inoculated to Anthurium spp. in this study, transmission to tobacco was never accomplished. In addition, transmissibility by white flies is not considered to be a property typical of other members of the potato Y virus group. Hence, it is concluded to be unlikely that Herold's virus and DMV are synonymous.

The relation of DMV to other aroid viruses is difficult to judge considering the general paucity of information provided by these previous studies. Raychaudhuri and Ganguly (56) for example reported that the aphid-transmissible "Chirke" disease of cardamon was manually transmissible to the aroid Acorus calamus. However, further information that would enable a correlation to be made between this virus and DMV was not provided. Also, in this study, neither cardamon

nor Acorus sp. were available for inoculation with DMV.

Dasheen mosaic virus was shown to have a wide host range within the Araceae. Although DMV infected species in 13 of the 16 aroid genera tested none of the inoculated 45 species of 17 other plant families became infected. These results, however, do not necessarily indicate that DMV is not synonymous with any other previously described members of the potato Y virus group. The plant species included in the host range were not all-inclusive with respect to all 103 members of the group listed by Edwardson (19).

James et al. (40) reported that DMV "occasionally" infected plants of T. expansa, although they apparently did not effect recovery of DMV back to any seedling aroid. Repeated efforts to infect T. expansa with DMV in this study were unsuccessful despite precautions taken to insure inoculum viability. If susceptible, T. expansa must be considered a marginal host, and certainly less satisfactory than seedlings of P. selloum as indicators for DMV.

No evidence for virus inactivators or inhibitors among aroids was noted in this study, as DMV was readily recovered to P. selloum seedlings. In addition to DMV, an isolate of cucumber mosaic virus from Commelina sp. manually inoculated to caladium seedlings was readily recovered to tobacco indicator plants (unpublished data of the author). The apparent lack of inhibitory substances is not surprising considering the study of Simons et al. (63) who showed that extracts of neither Dieffenbachia seguine nor Monstera deliciosa

contained substances inhibitory to the transmission of tobacco mosaic virus.

Since its recognition in 1969, DMV has been recovered from various locations including the United States (75), Europe (69, 75), the South Pacific (23,32,40,42), the Caribbean (2,42), South America (17) and Japan (3). In this study, specimens from India, Hawaii, the Fiji Islands, Trinidad and Guadeloupe were received, in addition to samples from Florida, and determined to be infected with DMV, thus confirming the international distribution of this virus. This widespread occurrence of DMV presumably reflects: 1) the antiquity of aroids as cultivated plants, 2) their obligatory means of vegetative propagation, 3) their extensive dissemination as popular cultivated plants and 4) the facility of DMV to be transmitted by aphids. The widespread occurrence of DMV in commercial caladiums is further abetted by certain circumstances peculiar to this industry. According to grower estimates, 96% of the world's caladiums are from Florida, 76% of which are produced in Highlands County on less than 600 acres (39). This crop is restrictively cultivated on acidic muck soil and with a minimum of crop rotation or isolation. Considering that the variety 'Candidum' comprises over 30% of the total production and that all these plants are derivatives of a single plant developed before the turn of the century (33), the ubiquity of DMV infections noted in this study for caladiums was seemingly inevitable.

This research shows DMV to be a serious pathogen of aroids.

Foliar symptoms are marked in some aroids, particularly 'Exotica' and 'Perfection' dieffenbachia. These symptoms in dieffenbachia merit grower concern, and, in some instances, entire beds have been abandoned due to foliar disfigurement. Symptoms in most aroids, however, are relatively inconspicuous -or absent altogether- and incited a minimum of concern, likely accounting for the relatively little attention devoted to aroid viruses. Of particular interest in this study was the intermittency of expressed symptoms for most aroids, but absence of apparent symptoms does not indicate recovery from virus. This fact was indicated in prolonged and controlled observations of several aroids, in particular cocoyam, 'Exotica' dieffenbachia, and P. selloum. In every instance, leaves with pronounced mosaic symptoms were expressed following periods when only symptomless leaves had developed. For this reason, it is concluded that most aroids naturally infected with DMV are unreliable indicators of infection. Several authors (10,12,23,40,41,42) have used vegetatively propagated aroids in virus studies, and concluded that symptoms expressed on inoculated plants indicated successful transmission. Such results should be viewed with caution unless workers can assure that plants were not already infected when inoculated. This investigation did not divulge reasons for the intermittency of symptom expression, although it was concluded that temperature was not likely to be a major contributing factor since leaves of 'Exotica' dieffenbachia expressed symptoms synchronously at two different temperature regimes. A

more likely factor in effecting symptom expression might be photoperiodic response. The author is not aware of published reports citing such a factor being responsible for intermittent symptom expression, although Reinert and Kasperbauer (57) have shown that the phytochrome system affects the multiplication of tobacco ringspot virus; and as reviewed by Butler and Downs (9), the phytochrome system has been implicated as having a major role in the development of photoperiodically sensitive plants. Accordingly, studies should be undertaken to ascertain the role of light in masking symptoms; the possibility exists that for certain foliage plants, symptoms can be masked by artificially manipulating day length. Similarly, such manipulation could also be employed on a practical basis to subdue virus multiplication prior to dissection of shoot tips for tissue culture, or, conversely, to augment virus multiplication to increase the reliability of DMV assays.

Quantitative losses due to DMV are more substantial than qualitative losses although they are probably far less apparent, when plants are ubiquitously infected. In controlled experiments with plants of caladium, dieffenbachia, P. selloum and Z. elliottiana, fresh plant weights were reduced as much as 63% and no less than 30%. Similarly, leaf areas were reduced 30 to 66% even when expressed foliar symptoms were not apparent.

These studies show conclusively that practical control of DMV is contigent upon the elimination of virus from propagating

stock. Two approaches for obtaining virus-free plants were given serious consideration in this study: seed propagation and shoot-tip culture. Alconero (1) attempted heat elimination of DMV from cocoyam but failed to inactivate the virus at temperatures the plant would withstand. Success with modification of this technique may be successful with other aroids, although relatively few viruses of the potato Y group have been controlled in this manner (52). Moreover, heat therapy is unlikely to completely eliminate other pathogens, including species of Pythium, Sclerotinia, Fusarium and Rhizoctonia known to be pathogenic to certain aroids.

Seed propagation is an effective means of eliminating most phytopathogens (4). Seed of many aroids were collected during this study (Table 1) and in no instance was there any evidence for seed transmission, even when seed was collected from plants known to be infected with DMV. Certain aroids, particularly P. selloum, are normally seed propagated, and resultant progeny closely resemble parental plants. As this study shows, however, progeny of some aroids, such as caladium and dieffenbachia, differ markedly from parental plants. For such aroids, therefore, this technique cannot be used on a practical scale to eliminate DMV from commercially desirable cultivars such as 'Candidum' caladium or 'Exotica' dieffenbachia. Nevertheless, results with caladium and dieffenbachia suggests a rich -but ignored- genetic potential for deriving improved horticultural varieties. For example, a need exists for "color" among foliage plants, such as that provided by the

golden green variegated pothos (Scindapsus aureus). A concerted breeding program with Dieffenbachia spp., could enrich the industry considerably by infusing it with color, as this study suggests. Similarly, horticulturalists have expressed interest in the possibility of developing less poisonous variants of dieffenbachia than are currently available (5).

The genetic potential of caladiums has previously been demonstrated as evidenced by the 2000 or more named varieties developed by such workers as Nehrling, Mead and Leitze (33). Whereas these evaluations were based principally upon foliage characteristics, incorporation of other factors such as cold-hardiness and disease resistance could be considered under the auspices of a future concerted breeding program.

Theoretically, shoot-tip culture affords the most ideal means of eliminating DMV and other phytopathogens while retaining the genetic integrity of the parental stock. In certain instances, this technique has the considerable added advantage as a means to mass propagate plants under aseptic conditions (48). This research resulted in successfully culturing in vitro caladium, cocoyam, C. cordata, C. nevillii and taro aseptically. This appears to be the first report of the in vitro culture of aroids. Interestingly, with the exception of the Cryptocoryne spp., the success reported herein involved plants of the tribe Colocasieae whereas failures involved other tribes in the Araceae. Although shoot-tips of both Cryptocoryne spp. differentiated into single plants which survived transfer to sand, neither species formed callus or proliferated

in culture indicating that the tissue culture techniques used in this study, while ideally suited for the Colocasieae, will have to be modified for members of other tribes. Attempts to culture dieffenbachia, A. modestum and P. selloum were not successful either because of contamination, or because explants failed to survive in culture. It is possible that proliferation for these plants could be effected through the use of growth regulators such as 2,4-D, as shown by Carter et al. (11) to be essential for the proliferation of oat callus.

Pathogen-free plants derived from tissue culture may effectively be used in pathogenicity trials with specific disease agents. Plants of 'Candidum' caladiums were used, for example, in determining that Pythium myriotylum was a significant root pathogen whereas three other species of Pythium were not (59).

Tissue culture may also be used as a valuable precautionary tool for those endeavoring to introduce exotic species or cultivars of caladium, cocoyam or taro into new areas. Leon (44) emphasized the dangers of inadvertently importing pathogens in vegetative plant parts and announced the need for defining areas where pathogen-free material could be obtained. Although some pathogens, such as DMV, may already be distributed world-wide, others may have a restricted range. Thus, culturing new accessions of these aroids in vitro, and screening plantlets before their release, would greatly minimize importing dangerous new pathogens accidentally.

The rapid proliferation of caladium, cocoyam and taro in

culture indicates that these plants can be propagated at an unprecidented rate. A newly developed caladium cultivar, for example, cannot be made available commercially until sufficient saleable stock can be produced which, according to grower estimates requires ca. 6-9 years during which time enough material has been accumulated to plant 0.5 acres with ca. 20,000 seed pieces. This study shows that at least 10-20 plantlets and 10-20 new cultures can be generated in vitro every three months. Realizing that each plantlet can attain maturity 6 months later, it would be theoretically possible to produce sufficient pathogen-free stock within three years to supplant the entire caladium industry of Florida consisting of an annual production of 40-50 million corms. A similar approach was considered for narcissus by Stone (64) who divulged the feasibility of replacing the entire narcissus stock of the Isles of Scilly with virus-free material.

Regardless of technique used to eliminate DMV and other pathogens from aroid planting stock, procedures should be adapted to prevent the re-establishment of pathogens. Presumably certification programs similar to those established for numerous other crops, such as potatoes and chrysanthemums, could be developed and standards appropriate for specific aroids established. Regretably, no such programs for aroids have been developed in Florida or elsewhere despite theireconomic importance. Ironically, DMV is firmly established in the cocoyam and taro accessions of the Federal Experiment Station at Mayaguez, Puerto Rico (2), and at the Saman Mocho Experiment

Station in Venezuela (17). Presumably higher standards of disease freedom should be initiated at research centers.

Once released, it is logical to assume that pathogen-free plants will become re-infected. This factor does not negate the wisdom of certification programs, however, and many, such as the bean seed program of New York, have withstood the test of time. Indeed, although DMV is aphid-transmitted, rates appear to be low and approximate the findings of Saladini and Zettler (61) for their observations of unexpectedly low incidences of sugarcane mosaic virus infections in St. Augustinegrass plantings in Florida. Zettler (personal communication) noted that only 2 of 112 and 34 of 157 Philodendron selloum seedlings became infected with DMV during test feedings (involving five aphids per test plant) by individuals of Aphis craccivora and Myzus persicae allowed single acquisition probes, respectively. These results are in marked contrast to the results with other potato Y type viruses of such plants as cowpea (73) and pepper (76), which have relatively high rates of aphid transmission.

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BIOGRAPHICAL SKETCH

Robert Dale Hartman was born in Miami, Florida on March 31, 1948. In June, 1966 he was graduated from Hialeah High School located in Hialeah, Florida. In September, 1966 he entered Miami Dade Junior College where he was awarded an A.A. degree in June, 1968. He subsequently entered the University of Florida where he received his B.S. degree in agriculture with majors in plant pathology and entomology and graduated with High Honors in June, 1970. He was supported in his upper division work by an Academic Scholarship and a National Defense Loan. He enrolled in the Graduate School of the University of Florida in the Department of Plant Pathology in January, 1971 and began work toward his Ph.D. degree in plant pathology with a minor in Ornamental Horticulture. His Ph.D. work was supported by a NDEA Title IV Fellowship and a departmental assistantship.

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He was married to the former Linda Diane Rockwell on October 25, 1969 and has a three year old daughter, Sandee Lynn.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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